30. (New) The soluble H4-1BB protein of claim 24 wherein the DNA molecule hybridizes to SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, or a combination thereof.

31. (New) The soluble H4-1BB protein of claim 24 wherein the DNA molecule encodes amino acid residues 1-186 of SEQ ID NO:2.

REMARKS

Reconsideration and withdrawal of the rejections of the claims, in view of the amendments and remarks presented herein, is respectfully requested. Claims 19-22 and 25 are canceled, and claim 27-31 are added. Claims 5 and 24 have been amended. The pending claims are 5-6, 24 and 26-31. The amendments to the claims are intended to clarify Applicant's invention and not to limit the scope of any equivalents to which the claims may be entitled.

Amended claims 5 and 24 are supported by originally-filed claim 5 and by Figure 2 of the specification.

New claims 27-31 are supported by originally filed claims 5, 7 and 16, and at page 14, line 25 through page 16, line 28, and Figures 1-2 of the specification.

The specification is amended at page 1, line 6 to indicate that at least one of the pending claims is entitled to the benefit of the filing date of U.S. application Serial No. 08/122,796, filed on September 16, 1963. This amendment addresses the Examiner's comment at item 5 of the final Office Action.

The Brief Description of Figure 1 is amended to refer to SEQ ID numbers so as to conform the specification to the requirements of 37 C.F.R. § 1.821(b).

In accordance with Rule 1.821(e), a copy of a substitute SEQUENCE LISTING in ASCII computer readable form was filed on October 26, 1998. It is certified that the contents of the paper version of the substitute SEQUENCE LISTING and the computer readable form thereof submitted on October 26, 1998 are the same. It is further submitted that the paper copy of the substitute

SEQUENCE LISTING and the computer readable form of the substitute SEQUENCE LISTING do not represent new matter.

35 U.S.C.§ 112, First Paragraph, Rejection

The Examiner rejected claims 5, 21-22 and 24-26 under 35 U.S.C. § 112, first paragraph. This rejection, as it may be maintained with respect to the pending claims, is respectfully traversed.

In particular, the Examiner alleges that the specification does not enable fragments of SEQ ID NO:2, e.g., fragments capable of binding a 4-1BB ligand, although the Examiner concedes that SEQ ID NO:2 is enabled by the specification.

Pending claims 5, 24 and 26-31 are directed to soluble forms of H4-1BB. At page 16 of the specification, Applicant describes the preparation of a construct that encodes a portion of H4-1BB containing the signal peptide and the entire extracellular domain of H4-1BB. To ascertain which amino acid residues of H4-1BB are encoded by the construct, the sequence of the oligonucleotides used to amplify this coding region (see lines 17-22 of page 16) can be aligned with the cDNA sequence of H4-1BB shown in Figure 2. Such an analysis indicates that residues 1-186 of H4-1BB contain the signal sequence and the entire extracellular domain of H4-1BB, i.e., the expression of these residues in a host cell results in a soluble form of H4-1BB.

Further, it is Applicant's position that one of ordinary skill in the art in possession of the present specification and knowledge generally available to the art would be apprised of how to determine whether the expression of a particular fragment of SEQ ID NO:2 results in a soluble polypeptide. Moreover, peptide synthesis, rather than recombinant DNA methods, can be used to prepare an array of polypeptides that have different portions of SEQ ID NO:2. It is certainly well within the skill of the art to metabolically label cells, e.g., with ^{35}S-methionine, which are transfected with an expression vector encoding a portion of H4-1BB, and analyze cell culture supernatants from transfected cells for the presence and size of labeled polypeptides relative to the presence and size of labeled polypeptides from control cell culture supernatants.

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In addition, Applicant's specification discloses that 4-1BB ligand is expressed on mature B cells and macrophage cell lines, but not on T cells (page 11). Thus, B cells, macrophage cell lines, and T cells can be used to screen fragments of SEQ ID NO:2, for example, soluble portions of SEQ ID NO:2, for their ability to specifically bind to B cells and/or macrophage and not to T cells. Alternatively, RNA is isolated from B cells or macrophage, and the isolated RNA used to prepare an expression library that can be screened with H4-1BB, or a soluble portion thereof, to identify clones that express a H4-1BB ligand. The Examiner is reminded that it is not necessary that a patent applicant make and test all the embodiments of his invention in order to meet the requirements of § 112. In re Angstadt, 190 U.S.P.Q. 214, 218 (C.C.P.A. 1976).

Moreover, with respect to the "undue experimentation" alleged by the Examiner to screen constructs encoding specific regions of H4-1BB, the fact that the outcome of such a synthesis/screening program is unpredictable is precisely why a screening program to locate biomolecules with target biological or physical properties would not be carried out by the art because the results cannot be predicted in advance.

In face, the Federal Circuit has explicitly recognized that the need, and methodologies required, to carry out extensive synthesis and screening programs to locate bioactive molecules do not constitute undue experimentation. In re Wands, 8 U.S.P.Q.2d 1400, 1406-1407 (Fed. Cir. 1988), the Court stated:

> The nature of monoclonal antibody technology is that it involves screening hybridomas to determine which ones secrete antibody with desired characteristics. Practitioners of this art are prepared to screen negative hybridomas in order to find one that makes the desired antibody.

Likewise, practitioners in the art related to the present application would be well-equipped to prepare and screen portions of SEQ ID NO:2 to identify those which are soluble and/or bind to a ligand. See also, Hybritech Inc. v. Monoclonal Antibodies Inc., 231 U.S.P.Q. 81, 84 (Fed. Cir. 1986) (evidence that screening methods used to identify characteristics [of monoclonal antibodies] were available to art convincing of enablement). Thus, the fact that a given claim

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may encompass a large number of polypeptides is not dispositive of the enablement issue, particularly in an art area in which the level of skill is very high and in which screening of large numbers of compounds has been standard practice for at least ten years (Ex parte Forman, 230 U.S.P.Q. 546 (Bd. App. 1986).

The Examiner is requested to consider the following documents as evidence that the art worker in possession of Applicant's specification and knowledge available to the art would be capable of identifying the ligand binding portion of a receptor, e.g., SEQ ID NO:2: Armitage et al. (Eur. J. Immunol., 22, 2071 (1992)), Linsley et al. (Science, 257, 792 (1992)), Smith et al. (Science, 248, 1019 (1990)), Mathews et al. (Cell, 65, 973 (1991)), and Miyamura et al. (J. Clin. Invest., 98, 1809 (1996)) (a copy of documents not submitted with the Rule 116 Amendment filed on February 26, 1999 are submitted herewith). Armitage et al. report that they used a biotin-labeled soluble fusion protein of CD40 (CD40 is a B cell membrane protein) and the Fc region of human IgG1 (CD40.Fc) to identify a CD40 ligand on a murine thymoma cell line (i.e., T cells). Murine thymoma cells which were selected for binding to the fusion protein expressed a soluble protein that stimulated human and murine B cell proliferation, an activity which could be neutralized by preclearing the supernatants with immobilized CD40.Fc. Thus, the authors concluded that they had identified a source of membrane-bound and soluble CD40 ligand.

Linsley et al. disclose that B7 is a molecule on antigen presenting cells that binds to T cell surface molecules CD28 and CTLA-4. They report that a soluble fusion protein having the extracellular domain of CTLA-4 and Ig blocked the binding of B7 to CD28. Thus, the portion of CTLA-4 in the fusion protein binds to ligand.

To isolate the receptor for TNF-α and TNF-β, Smith et al. employed the radiolabeled ligand TNF-α as a probe to screen an expression library prepared from human lung fibroblasts. The cloned receptor, TNFR, was introduced into COS cells and shown to have the same ligand binding properties as the native receptor.

^{125}-activin was employed to screen COS cells transfected with cDNAs from AtT20 mouse cells to isolate an activin receptor (Mathews et al.). In the Introduction section of the Mathews et al. paper, the authors note that a number of receptors, including the erythropoietin, IL-4, IL-6, IL-7,

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interferon-γ, GM-CSF and G-CSF receptors, have been cloned based on their ability to bind a labeled ligand following expression of a cDNA library in mammalian cells.

Miyamura et al. describe the preparation and screening of a series of deletion constructs encoding portions α-galactosidase A. Thus, it is clearly within the skill of the art worker to manipulate constructs, e.g., by deletion analyses, to identify constructs that encode a portion of a polypeptide and compare the activity of that polypeptide activity, e.g., the ligand binding activity, to that of the full length polypeptide.

Therefore, given Applicant's disclosure of the amino acid sequence of H4-1BB, i.e., SEQ ID NO:2, and the amino acid residues comprising the extracellular domain, and the skill of the art worker in the relevant art area, it is Applicant's position that the preparation and screening of fragments of SEQ ID NO:2 to identify regions of SEQ ID NO:2 that are soluble and/or bind to a cell membrane ligand is well within the skill of the art.

The Examiner alleges that Loo et al. (J. Biol. Chem., 272, 6448 (1997)) provide evidence that it would require undue experimentation to identify the ligand for H4-1BB. Loo et al. disclose that mouse 4-1BB binds to mouse 4-1BBL (L=ligand) and rat laminin (LN) and that human 4-1BB binds to human 4-1BBL but not to rat LN. Significantly, Loo et al. state that while it is unlikely, "we cannot rule out the possibility that expression of h4-1BB in a heterologous mammalian expression system (COS cells instead of T cells) selectively affected the ability of h4-1BB to bind extracellular matrix proteins" (sentence bridging pages 6454-6455). In addition, the extracellular matrix protein employed in the extracellular matrix binding assay was that of rat. Therefore, the lack of binding of human 4-1BB to "extracellular matrix protein" may be due to the amino acid sequence differences between rat and human LN. Thus, there is nothing in the Loo et al. article that supports the proposition that it would require undue experimentation to identify the ligand(s) for H4-1BB.

With respect to the enablement of claim 26, the Examiner asserts that the specification provides (a) no dosage information, (b) no guidance as to how to make pharmaceutical formulations, (c) no reasonable expectation that the formulations would treat a disease or condition, e.g., suppress the immune system during organ transplantation, (d) which autoimmune diseases are to be treated, or (e) how to select enhancing versus suppressing activity.

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Claim 26, as amended, is dependent on claim 24 and is directed to a pharmaceutical composition comprising a soluble H4-1BB polypeptide which comprises the extracellular domain of SEQ ID NO:2, or a fragment thereof, in admixture with a suitable diluent, carrier or excipient.

The Examiner also asserts that the specification does not provide a reasonable expectation that a pharmaceutical composition comprising a soluble H4-1BB polypeptide which comprises the extracellular domain of SEQ ID NO:2, or a fragment thereof, in admixture with a suitable diluent, carrier or excipient (claim 26) would be effective to treat T cell-mediated immune responses. Figures 4(b)-(c) and 5(b)-(c) of the specification illustrate the interaction of 4-1BB with its ligand, and how 4-1BB can be used to suppress T cell-dependent immune responses. See also pages 17-18.

With respect to (c)-(e), the specification discloses and illustrates that 4-1BB can be used to suppress T cell-dependent immune responses. See pages 17-18 and Figures 5(a)-(c). Since 4-1BB production is induces during T cell activation, blocking the interaction of T cells with antigen presenting cells which express H-1BB ligand by contacting cells with a polypeptide such as that recited in claim 24 will lead to immunosuppression.

Moreover, one of ordinary skill in the art is capable of determining whether a disease is associated with a specific immune cell, e.g., a B cell or a T cell, and whether that disease is associated with aberrant stimulation or suppression of a particular immune cell (see, for example, U.S. Patent No. 5,645,820 which discloses autoimmune diseases associated with B cells and autoimmune diseases associated with T cells). Thus, if the proliferation of T cells in a particular disease or pathology is excessive, the suppression of those T cells would be indicated. Moreover, it is within the skill of the practitioner to use *in vitro* assays, or an animal model, to test an agent for its ability to stimulate or suppress an immune response.

The Examiner is also requested to note that the reaction of T cell receptors, such as isotypes of the leukocyte common antigen, with blocking or "anergizing" ligands such as anti-CD45R monoclonal antibodies has been shown to be effective in animals models to reverse transplant rejection. See, for example, WO 96/32965 ("CD45RB Binding Compounds for the Prevention of Transplant Rejection"; Zheng et al., Transplant. Proc., 27 389 (1995); and Lazarovits, Transplantation, 54, 724 (1992).

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To provide further evidence that the specification, in view of the skill of the art worker in the relevant art area, enables the use of the claimed composition to treat T cell-mediated immune responses, the Examiner is requested to consider Linsley et al. (Science, 257, 792 (1992)). Linsley et al. disclose that B7 is a molecule on antigen presenting cells that binds to T cell surface molecules CD28 and CTLA-4 (see also Figures 4(a)-(b) and 5(a)-(b) of the specification). The binding of B7 on B cells to CD28 or CTLA-4 on T cells activates T cells. A soluble fusion protein having the extracellular domain of CTLA-4 and Ig suppressed T cell-dependent antibody responses *in vivo* after mice were injected with one of two different antigens.

Since 4-1BB production is induced during T cell activation, blocking the interaction of T cells with antigen presenting cells which express H4-1BB ligand by contacting cells with a polypeptide such as that recited in claim 26 will lead to immunosuppression. Thus, if the proliferation of T cells in a particular disease or pathology is excessive, the suppression of those T cells would be indicated.

With respect to (a) and (b), it is Applicant's position that the selection of dosages and the preparation of pharmaceutical formulations is well within the skill of the art, and is necessarily empirical and patent-dependent. (See, In re Johnson, 282 F.2d 370, 127 U.S.P.Q. 216 (C.C.P.A. 1960) (the selection of suitable dosages is within the skill of the art)). Methods for extrapolating from dosages effective in animals to dosages effective in humans are known to the art. See, for example, U.S. Patent No. 5,294,430. Applicant need not teach, and preferably omits, that which is known to the art. Hybritech Inc. v. Monoclonal Antibodies Inc., 231 U.S.P.Q. 81, 84 (Fed. Cir. 1986).

With respect to pharmaceutical formulations, the specification notes that lymphokines, e.g., interferons, interleukins, erythropoietin, and tumor necrosis factor, have been produced for therapeutic use (page 2). Moreover, the Examiner has acknowledged that "[p]harmaceutical compositions consisting of secreted forms of lymphocytic proteins are common to the art" (at page 8 of the Office Action dated a April 19, 1996). Further, soluble murine 4-1BB was administered to a rat to prepare monoclonal antibodies specific for 4-1BB (Pollock et al., J. Immunol., 150, 771 (1993)). Thus, it is well within the skill of the art worker, in possession of

Applicant's specification and knowledge available to the art, to prepare pharmaceutical compositions comprising H4-1BB or a portion thereof and select appropriate dosages.

It is respectfully submitted that the pending claims are in conformance with the requirements of 35 U.S.C. § 112, first paragraph. Hence, the Examiner is requested to withdraw the § 112(1) rejection of the claims.

The 35 U.S.C.§ 103 Rejection

The Examiner rejected claims 5-6, 21-22 and 24 under 35 U.S.C. § 103(a) as being unpatentable over Schwarz et al. (GenBank Accession No: L12964) in view of page 45 of Ayala et al. (Mod. Genetics, Benjamin Cummings Publ. (1980)). As this rejection relates to the pending claims, it is respectfully traversed.

Schwarz et al. disclose the nucleotide sequence encoding, and the inferred amino acid sequence of, ILA. The inferred amino acid sequence of ILA has one amino acid substitution relative to Applicant's SEQ ID NO:2. The substitution is at amino acid position 107.

The Examiner is respectfully requested to consider the Rule 131 Declaration, filed on February 26, 1999, which was executed by Dr. Byoung Kwon, the inventor of the present application. In the Rule 131 Declaration, Applicant declares and documents that in the United States, he had conceived of isolating and purifying DNA encoding human 4-1BB prior to the April 22, 1993 publication date of Schwarz et al. Moreover, in the Declaration, Applicant declares and documents that, after conception, he proceeded diligently to reduce the invention to practice in the United States. In particular, Applicant refers to Exhibits A, B, C, and D, attached to and incorporated by reference into the Declaration, as factual evidence of conception of the invention prior to the effective date of Schwarz et al. coupled with due diligence from conception to the filing of a patent application.

Exhibit A is a photocopy of certain pages from U.S. application Serial No. 08/012,269, filed on February 1, 1993, an application of which Applicant is the sole inventor. U.S. application Serial No. 08/012,269 discloses the nucleotide sequence and inferred amino acid sequence of murine 4-1BB (page 17, and Figures 2A and 2B). At page 24, a method of isolating

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the human homolog of murine 4-1BB is disclosed. The preparation of a soluble form of murine 4-1BB and a murine 4-1BB fusion protein is described at pages 29 and 70, respectively. The introduction of a construct encoding soluble murine 4-1BB into host cells, and the subsequent purification of soluble murine 4-1BB, is disclosed at page 29.

Therefore, prior to the effective date of Schwarz et al., Applicant had prepared recombinant murine 4-1BB polypeptide, including a soluble, murine 4-1BB fusion polypeptide. Moreover, Applicant had envisioned methods to isolate the human homolog of murine 4-1BB, an obvious variation of murine 4-1BB.

Exhibit B and Exhibit C are each a photocopy of an autoradiogram. Various combinations of degenerate primers (such as those described at pages 14-15 of the present specification which are complementary to nucleotide sequences in the extracellular domain of 4-1BB) and human lymphocytic RNA were employed in a reverse transcriptase-polymerase chain reaction to obtain amplification products that corresponded to the human homolog of murine 4-1BB. The DNA products were separated on agarose gels and DNA isolated from individual bands. The isolated DNAs were subjected to Southern blot analysis using a radiolabeled murine 4-1BB DNA probe under low stringency conditions, the results of which were recorded on an autoradiogram (Exhibit B). The DNA in the hybridizing band in lane 7 of Exhibit B was cloned and then subjected to Southern blot analysis using radiolabeled murine 4-1BB DNA, the results of which were recorded on another autoradiogram (Exhibit C). Exhibit C is dated prior to the effective date of Schwarz et al.

Thus, the combination of Exhibits A, B, and C evidence Applicant's conception of the invention prior to the effective date of Schwarz et al.

Exhibit D demonstrates that the invention disclosed in Exhibits A, B and C was diligently pursued from a time before the effective date of Schwarz et al., i.e., April 22, 1993, to a time approximately five months after the effective date of Schwarz et al. by the filing of the parent application to the present application.

The present claims are directed to an isolated H4-1BB polypeptide comprising SEQ ID NO:2, or a soluble portion thereof. The Examiner is reminded that Applicant need demonstrate

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only so much of the claimed invention as taught by the prior art reference, or what is rendered obvious in view of the reference. In re Stempel, 113 U.S. P.Q. 77 (C.C.P.A. 1957). Thus, the enclosed Rule 131 Declaration properly establishes Applicant's date of invention as earlier than the effective date of Schwarz et al. Therefore, Schwarz et al. cannot be used to support a rejection of the claims under 35 U.S.C. § 103(a), and so the Examiner is respectfully requested to withdraw the § 103(a) rejection of the claims.

Even if, assuming for the sake of argument, Schwarz et al. is available as a reference against the present claims, Schwarz et al. do not disclose or suggest preparing an expression vector with their disclosed cDNA, much less which portion, if any, of the polypeptide encoded by the ILA cDNA is extracellular. Thus, this reference does not render Applicant's invention obvious.

Page 45 of Ayala et al. discloses that many, and possibly all, genes have multiple alleles. Nevertheless, there is nothing at page 45 of this reference that teaches or suggests the preparation or isolation of a polypeptide having SEQ ID NO:2 or a fragment thereof. Thus, Applicant's invention is not rendered obvious by Ayala et al.

To establish a *prima facie* case of obviousness, three basic criteria must be met. First, there must be some suggestion or motivation, either in the cited references themselves or in the knowledge generally available to an art worker, to modify the reference so as to arrive at the claimed invention. Second, there must be a reasonable expectation of success, i.e., that the invention would be operable. Finally, the prior art reference must teach or suggest all the claim limitations (M.P.E.P. §2143).

As discussed above, neither cited reference discloses or suggests the isolation of a variant of ILA, or the preparation of a soluble form of H4-1BB, much less which portion of the native H4-1BB polypeptide is extracellular, e.g., residues 1-186 of SEQ ID NO:2. Hence, the combination of Schwarz et al. and Ayala et al. does not render Applicant's invention obvious.

Based on the discussion above, the Examiner is respectfully requested to withdraw the § 103(a) rejection of the claims.

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CONCLUSION

Applicant believes the claims are in condition for allowance and request reconsideration of the application and allowance of the claims. The Examiner is invited to telephone the below-signed attorney at (612) 373-6959 to discuss any questions which may remain with respect to the present application.

Respectfully submitted,

By their Representatives,

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